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Canadian Journal of Research

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LIQUID FLOW AT SMALL CONSTANT RATES[1]

BY R. O. KING[2] AND R. R. DAVIDSON[3]

Abstract

A supply of liquid at an extremely small rate is frequently required for laboratory scale experiments. An unusually convenient method of supply became possible on its becoming known that Faraday's law held good even for electric currents of the order of microamperes, if electrodes were properly placed in a suitable electrolyte. The apparatus required is simple. Gas is liberated from the electrolyte in a small cell at a rate proportional to the current passing through it. The cell is connected to a vessel containing the liquid to be metered and when temperature and pressure become steady, liquid is discharged through an outlet from the vessel at a rate directly proportional to the electric current through the cell.

Introduction

It became necessary in the course of flow method oxidation experiments in the Colloid Science Laboratory, Cambridge University, to provide mixtures of air with liquids such as pentane, in known proportions, at a rate of flow as small as 25 cc. per min., liquid flow then being required at the rate of about 0.002 gm. or 1/20 of an ordinary drop per min. The commonly used methods of supplying liquid at very small rates of flow are somewhat complicated and inflexible and it was suggested by Prof. E. K. Rideal that the addition liquid be expelled from the container at the required constant rate by electrolytic gas evolved in a connected vessel by electricity metered accordingly. Two types of "Microdoser" were developed. They made possible an exceptional rapidity and accuracy of experiment.

Microdoser, Type A

This is suitable for liquids of low vapour pressure. The type is illustrated in Fig. 1. The gas evolved in the cell forces the contents of the fuel vessel into the air stream at a rate directly proportional to the electric current passed through the cell under steady conditions of temperature and pressure (Faraday's law). The law is known to apply generally, but when the electric current is of the order of microamperes it is necessary to use an electrolyte other than dilute sulphuric acid and electrodes sufficiently separated to prevent mutual depolarization. The electrodes were of platinum and $\frac{1}{2}$ in. apart in the cell as constructed, and the electrolyte, a solution of barium hydroxide in water (8.6 parts per 1000).

[1] *Manuscript received May 12, 1943.*
Work carried out at the Colloid Science Laboratory, Cambridge University, England.
[2] *Present address: 260 Metcalfe St., Ottawa, Canada.*
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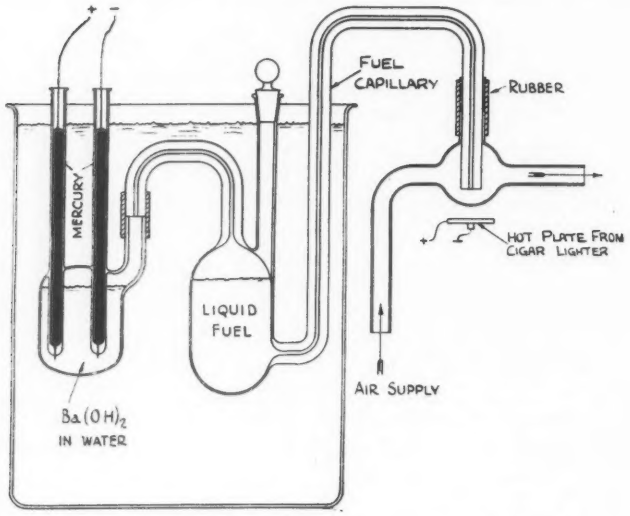

FIG. 1. *Rideal microdoser, Type A. Scale: about one-half.*

The electrolytic gas evolved in the cell becomes saturated with the vapour of the liquid being metered. The correction required can be reduced to negligible proportions, in some cases, by suspending the microdoser in water–ice, as shown by the figure.

Microdoser, Type B

This type, illustrated in Fig. 2, is suitable especially for metering liquids of relatively high vapour pressure such as pentane, which are insoluble in water, a vapour pressure correction being rendered unnecessary by interposing a water seal between the liquid in the electrolytic cell and that to be metered. The design has the further advantage that the possibility of gas leak is avoided.

A current of 1.113 ma. through the cell is required to add pentane to air at 20° C. and normal pressure, flowing at the rate of 100 cc. per min. if a combining proportions mixture, $8\,O_2 + C_5H_{12}$, is required. The current need only be varied proportionately to provide similar mixture proportions at other rates of air flow or to vary the proportion of pentane added at any rate of air flow.

When the Microdoser is used to deliver a volatile liquid such as pentane at a rate of about 0.5 gm. per hour, a drop forms on the end of the capillary projecting into the air stream and the size reaches equilibrium when the rate of evaporation becomes equal to the rate of supply of liquid to the drop. At still lower rates of liquid flow an equilibrium meniscus forms in the capillary. Equilibrium changes with change in rate of air or liquid flow, and an indication of when steady conditions are reached is obtained accordingly. When the rate of liquid flow is so great that drops fall from the end of the capillary,

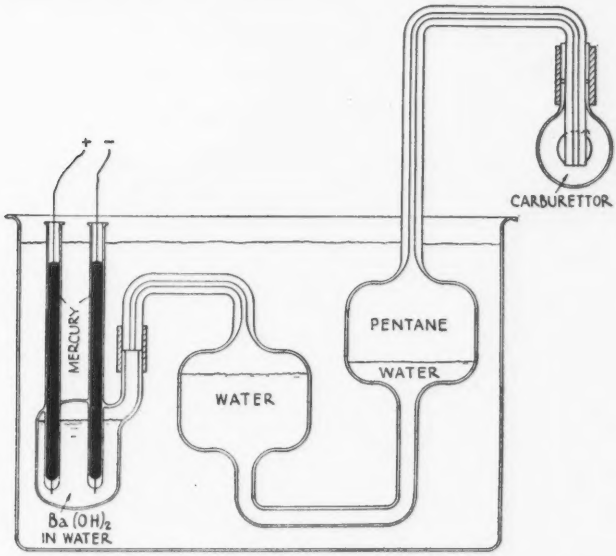

FIG. 2. *Rideal microdoser, Type B. No correction required for vapour pressure.*

recourse is had to the usual cotton wool device, and if the liquid is of low vapour pressure, a small electric hot plate has been used as shown in Fig. 1.

The barium hydroxide electrolyte should be replaced by fresh solution after an interval of time depending on extent of use (ampere-hours) but extending generally to months.

Acknowledgments

The thanks of the authors are due to Prof. Rideal for suggesting the idea of the Microdoser and to the sponsors of the research at Cambridge for which the device was a necessity.

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VOL. 21, SEC. B. JULY, 1943 NUMBER 7

A COLORIMETRIC METHOD FOR THE DETERMINATION OF FAT-PEROXIDES AND ITS APPLICATION IN THE STUDY OF THE KEEPING QUALITY OF MILK POWDERS[1]

BY R. A. CHAPMAN[2] AND W. D. MCFARLANE[3]

Abstract

A colorimetric method based on the oxidation of ferrous to ferric iron and the determination of the latter as ferric thiocyanate has been found suitable for the estimation of fat-peroxides in milk powder. To an acetone extract of milk powder is added a solution consisting of 0.1% of ferrous ammonium sulphate and 0.4% of ammonium thiocyanate in 96% acetone, and the colour is developed by heating. The intensity of the red colour is measured with a Coleman spectrophotometer and is found to bear a close relation to the keeping quality of the milk powder. Peroxide values determined by this method are considerably higher than those obtained by an iodimetric procedure (4).

Introduction

The numerous methods that have already been proposed for the detection and estimation of oxidative rancidity in fats and fatty foods have been reviewed by Lea (2). Most of these methods are of doubtful value for the examination of a material such as milk powder, in which the fat is intimately associated with the protein and can be extracted only by procedures that are liable to decompose the fat-peroxides. However, Smith (4) has adapted the iodimetric methods to the estimation of peroxides in milk powder. All iodimetric procedures can be criticized for the slow liberation of the iodine (5) and the possibility of its reabsorption by the fat.

The writers have studied the keeping quality of milk powders during storage, comparing the results obtained by Smith's (4) method and organoleptic tests, and have noted that the powders may have a tallowy taste and odour for some time before a measurable quantity of peroxides has been formed. The off-flavours are not developed in powders stored in nitrogen, hence must be due to oxidation.

Apparently, therefore, Smith's (4) method is not sufficiently sensitive to detect incipient oxidative changes. Hollender and Tracy (1) have arrived at a similar conclusion with reference to the limitations of this method for detecting early oxidation of the fat in milk powder.

[1] *Manuscript received in original form March 22, 1943, and as revised, April 29, 1943. Contribution from the Department of Chemistry, Faculty of Agriculture (McGill University), Macdonald College, Que. Macdonald College Journal Series No. 181.*

[2] *Research Assistant in chemistry.*

[3] *Professor of Chemistry.*

Peroxides in gasoline have been estimated by Yule and Wilson (7) and by Young *et al.* (6) using a method based on the oxidation of ferrous to ferric iron by the peroxides present and the colorimetric determination of the ferric iron as ferric thiocyanate. The procedure described below is essentially an adaption of this method in which acetone is used as the solvent. Results are presented to indicate its possibilities as a criterion of the keeping quality of milk powders.

Procedure

I. Preparation of the Reagent

The reagent consists of a solution of 0.1% of ferrous ammonium sulphate and 0.4% of ammonium thiocyanate in 96% acetone. The ammonium thiocyanate is weighed into a volumetric flask and an amount of distilled water equivalent to 4% of the final volume is added. The salt is allowed to dissolve and the flask almost filled with anhydrous acetone. The ferrous ammonium sulphate is added, and the solution diluted to the mark, and shaken thoroughly. The reagent is held in the dark for two hours, shaking frequently during the interim. After filtering through an acetone washed filter paper, the reagent is ready for use.

If prepared as outlined, the reagent will have a faint pink tinge and should give a reading of 80 to 90 on the Coleman spectrophotometer with the instrument set at 485 mμ. It must be kept in the dark in order to retard a gradual increase in the intensity of the colour Even when the reagent was protected, the colour increased to some extent, but it was found that the same peroxide values were obtained with a milk powder when using reagents that gave readings of from 60 to 90 on the galvanometer. The reagent may be used for three or four days, although the range of the determination is considerably

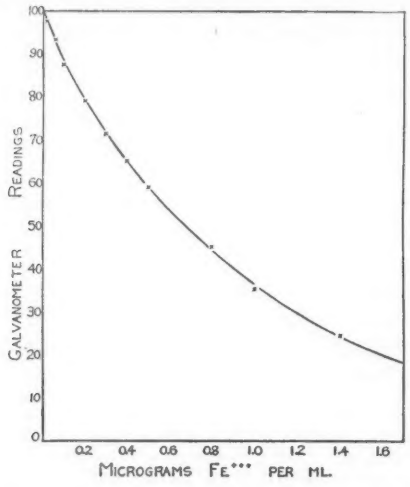

FIG. 1. *Calibration curve of ferric thiocyanate in acetone.*

restricted if the colour in the blank becomes too intense. Acetone which has not already been used in the test must be carefully purified by distilling it from a small amount of ferric chloride, drying over calcium chloride, and redistilling before use. Spent solvent does not require any addition of ferric chloride since ferric ions are already present, but it must be carefully dried and redistilled since the water content is critical.

II. Preparation of the Calibration Curve

A standard of reference curve (Fig. 1) is prepared from readings obtained with standard solutions of ferric chloride (0.2 to 14.0 μg. per ml.) in purified anhydrous acetone. A 1 ml. aliquot of the solution is added to 9 ml. of reagent consisting of 0.4% of ammonium thiocyanate in 96% acetone. The intensity of the colour is measured with a Coleman spectrophotometer set at 485 mμ. The colour, which develops without heating, fades slowly so that the readings should be taken at once. The reagent, which must be colourless, is used as a blank in the initial setting of the instrument.

III. Method

A 200 mg. sample of finely divided milk powder is transferred to a 22 by 150 mm. test-tube and 8 ml. of anhydrous acetone is added. A small water condenser is fitted into the neck of the tube and the suspension is refluxed for 20 min. on a water-bath at 65° to 70° C. The tube is removed from the bath, cooled, and the extract filtered through an acetone-washed filter paper into a glass-stoppered 10 ml. graduated cylinder. The residue in the tube is washed with 1 ml. of acetone, poured on to the filter, and again washed with 1 ml. of the solvent. The filtrate is diluted to volume with acetone.

The remainder of the determination is carried out under red light since sunlight or strong electric light intensifies the colour. If only comparative results are desired, this precaution should not be necessary. A 9 ml. aliquot of the reagent is transferred to a test-tube, 1 ml. of acetone extract of the milk powder added and the contents of the tube thoroughly mixed. The tube is heated first at 70 to 80° C. until the first evolution of gas bubbles occurs and then for 10 min. at 50° C. The intensity of the developed colour is determined, as described above, in the Coleman spectrophotometer using pure acetone as a blank. A reading is also made with 9 ml. of the reagent treated in a manner identical with the test. The difference between the values for the determination and the reagent blank gives the ferrous iron oxidized to ferric iron by the peroxides in the milk powder extract. Total peroxides may be calculated as follows:—

$$\frac{A \times B}{C \times 55.84} = \text{milliequivalents of peroxide per kilogram of milk powder,}$$

where

A = Micrograms of Fe^{+++} in 10 ml. of test solution minus micrograms of Fe^{+++} in 9 ml. of reagent blank,

B = Volume of extract (10 ml.),

C = Wt. of sample, in grams,

55.84 = Equivalent weight of iron.

Discussion

Considerable difficulty was encountered in finding a solvent that would satisfactorily extract the fat from milk powder. Absolute methyl alcohol (6) and 50% acetone (7) were not suitable, nor was diethyl ether, petroleum ether, or chloroform. Absolute acetone, however, was found to remove almost all the fat if the refluxing were continued for several hours. It was noted that although the percentage of the fat extracted increased during this period, the peroxide value of the milk powder reached a maximum after 20 min. and further refluxing gave no higher values. A 200 mg. sample was found to be the most satisfactory from the standpoint of efficiency of extraction, and it also permitted a considerable range of oxidative rancidity before it became necessary to dilute the milk powder extracts to bring the intensity of the colour into the range measured by the spectrophotometer. If the milk powder was thoroughly mixed there was no appreciable sampling error.

Although the supernatant liquid was perfectly clear after refluxing, it was necessary to filter the extracts before carrying out the test on an aliquot since the residue had some reducing action and, if allowed to remain in the solution, it caused fading of the colour. The optimum concentration of ammonium thiocyanate was found to be 0.4% although a range of 0.2% to 0.75% showed only slight variation. A concentration of ferrous ammonium sulphate of 0.1% gave a maximum reading and the colour was comparatively stable. It was noted, however, that the presence of water in the acetone had a marked effect on the reaction. Absolute acetone gave maximum colour but rapid fading. The addition of 10% of water to the acetone improved the stability but reduced the colour to only 50% of the former value. The writers finally selected 96% acetone as the optimum for sensitivity and stability. It is imperative that all glassware used in the test be scrupulously clean. Hot concentrated nitric acid has been found satisfactory as a cleaning agent.

Application of the Method

Previous work in this laboratory had indicated that wheat germ oil alone and in combination with other antioxidants had a definite stabilizing effect on spray-dried whole milk powders. However, these conclusions had been reached chiefly through organoleptic tests, since chemical methods outlined in the literature were not sufficiently sensitive to detect the slight changes that occur during the induction period. It was felt that with the much more sensitive ferric thiocyanate method it might be possible to follow these chemical changes.

Therefore two samples of spray-dried whole milk powder were prepared. One sample was dried without any treatment, but to the second was added an amount of modified wheat germ oil* equivalent to 0.1% of the butterfat. The stabilizing agent was added to a small quantity of skim-milk, and the mixture was homogenized, added to the whole milk, and thoroughly mixed,

* *Wheat germ oil antioxidant—Formula C, Viobin (Canada) Ltd., Montreal.*

and then it was spray-dried by means of standard commercial equipment. These two samples were held at room temperature in partially filled friction top cans and opened periodically for examination. These conditions were conducive to the development of oxidative rancidity in the milk powder. The results of the analysis are given in Fig. 2.

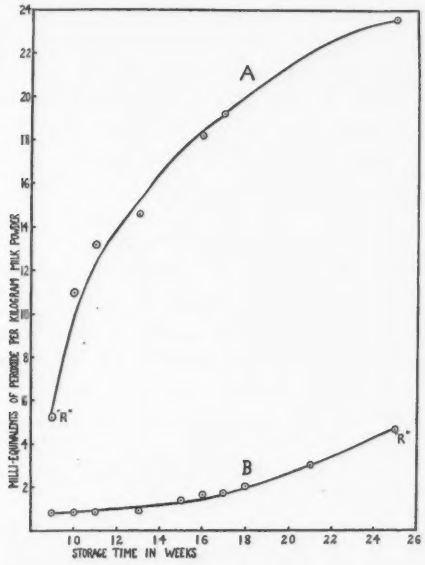

FIG. 2. *The relative rates at which peroxides are formed in spray-dried whole milk powders made from (A) untreated milk and (B) milk treated with wheat germ oil antioxidant. "R" indicates the point at which rancidity was first detected by the taste and odour of the reconstituted milk.*

It is evident from these results that the addition of the wheat germ oil antioxidant had a marked effect on the keeping qualities of the powders. At nine weeks the untreated sample had a definitely unpleasant taste and odour, and gave a strong test for oxidative rancidity. The same stage of deterioration was not reached by the treated powder until it had been stored for 24 weeks. The treated powder was tested for peroxides after 20 weeks' storage by the Smith (4) iodimetric method, but it gave a negative test although the powder had apparently begun to deteriorate, since the intensity of the ferric thiocyanate colour was increasing.

To determine the relation between the peroxide values as obtained by the two methods a series of spray-dried powders were examined with the results shown in Table I.

It is evident from the results that peroxides can be detected at an earlier stage of deterioration by the method outlined in this paper and the

TABLE I

PEROXIDE VALUES OF MILK POWDERS AS DETERMINED BY IODIMETRIC AND THIOCYANATE METHODS

Samples	Milliequivalents of peroxide per kilogram	
	Iodimetric method (4)	Thiocyanate method
1. Freshly opened vacuum-packed tin	0	0
2. Same sample; stored 3 weeks in open tin at room temp.	0	0.45
3. Sample *B* (Fig. 2) at 16 weeks' storage	0	2.30
4. Sample *A* (Fig. 2) at 16 weeks' storage	6.76	21.4
5. Rancid sample—1 year old	17.0	51.8

values obtained are considerably higher. This is not to be expected since potassium iodide is more easily oxidized than ferrous iron.

It was considered that this behaviour might have been due to an error in the calibration curve, the method of extraction of the fat, or to the destruction of peroxides by the manner in which glacial acetic acid is used in Smith's (4) procedure. The accuracy of the calibration curve was confirmed by standardizing a solution of hydrogen peroxide with permanganate and then determining its normality by the colorimetric procedure. Three determinations on a 9.5×10^{-5} *N* solution of hydrogen peroxide gave an average value of 9.8×10^{-5} *N*.

In order to check the efficiency of the extraction procedures the following experiment was carried out. A 10 gm. sample of spray-dried whole milk powder was refluxed for 20 min. with 100 ml. of anhydrous acetone, filtered, the residue washed, and the filtrate made up to volume in a 100 ml. volumetric flask. Two 25 ml. portions of this extract were evaporated down to dryness *in vacuo* at 45° C., and 25 ml. of glacial acetic acid was added. They were allowed to stand for five minutes and 25 ml. of chloroform was added, and the peroxides were then determined iodimetrically (4). Two 5 ml. aliquots were also evaporated to dryness under conditions identical with those employed with 25 ml. aliquots, the residue was dissolved in anhydrous acetone, and peroxides were determined by the thiocyanate method. The iodimetric method gave values of 3.58 and 3.66 milliequivalents of peroxide per kilogram of milk powder as compared to 12.7 and 12.4 by the thiocyanate procedure.

It is therefore clearly indicated that the extraction of the fat is not the cause of the discrepancy, but that the low values obtained by the iodimetric method are possibly due to destruction of the more unstable peroxides by the glacial-acetic-acid–chloroform reagent. The peroxide of oleic acid is known to be decomposed quite easily, while linoleic acid peroxide is considerably more stable (3). There should therefore be a greater discrepancy in peroxide values

as determined by the two methods when applied to the oxidation product of oleic acid as compared to the oxidation product of linoleic acid.

Two 5 gm. samples of oleic and linoleic acid were dissolved in 100 ml. of chloroform and oxygen was bubbled through the solutions for 48 hr. Aliquots were taken down to dryness by vacuum distillation at 45° C. and the peroxide values determined. Oleic acid gave values of 12.5 and 67.3, and linoleic acid, 529 and 1329 milliequivalents of peroxide per kg. by the iodimetric and thiocyanate methods respectively.

It would therefore appear that the lower values obtained by the iodimetric technique can be attributed to the breakdown of unstable peroxides in the presence of glacial acetic acid. The thiocyanate procedure is carried out in acetone solution at pH 5.6 to 5.9, and apparently under these conditions the compounds responsible for the test are much more stable.

Acknowledgments

This investigation was sustained in part by a grant from the National Research Council of Canada. The writers are indebted to Dr. A. R. M. McLean, Eastern Dairies Limited, Montreal, for preparing the powdered milk samples, and to Viobin (Canada) Limited, Montreal, for kindly supplying the wheat germ oil antioxidant.

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THE ALKALOIDS OF PAPAVERACEOUS PLANTS

XXXVIII. *BOCCONIA ARBOREA* WATS.[1]

By Richard H. F. Manske[2]

Abstract

Bocconia arborea Wats. has yielded chelerythrine, protopine, allocryptopine, and a new base, alkaloid P61, probably isomeric with and closely related to chelerythrine. Phenolic alkaloids were absent but three neutral nitrogenous compounds were obtained. They are referred to as Compound A ($C_{20}H_{17}O_4N$), Compound B ($C_{20}H_{15}O_4N$), and Compound C ($C_{31}H_{33}O_5N$).

The genus *Bocconia* L., containing nine species, is endemic from Mexico to northern South America and adjacent islands. It is closely related to but distinct from the Asiatic *Macleaya* R. Br. with which it has nevertheless been confused. Both genera are closely related to the North American *Sanguinaria canadensis* L. and it is, therefore, no accident that the contained alkaloids are similar or identical. *B. arborea* Wats., the subject of the present communication, is the only truly arborescent plant in the entire Papaveraceae family, although the genera *Romneya* Harv. and *Dendromecon* Benth. include shrubs and near dendritic forms.

The chief alkaloid proved to be chelerythrine, which was readily obtained in a state of purity. Sanguinarine, hitherto always associated with chelerythrine, appears to be absent. There is nevertheless present a small amount of a base (P61) which appears to be closely related to the other phenanthrene isoquinoline alkaloids. It is isomeric with chelerythrine ($C_{21}H_{19}O_5N$), and a methoxyl determination shows the presence of three such groups, but one of these probably comes from an N-methyl source because chelerythrine itself showed three groups whereas only two are known to be present. It is not improbable therefore that alkaloid P61 has the structure of chelerythrine with the methylenedioxy group and the methoxyl groups interchanged. Two more known alkaloids, namely, protopine and allocryptopine were present, but no phenolic bases could be found. The presence of other bases except in minute amounts is unlikely, since all but a small amount of the total alkaloids was obtained crystalline.

There were present however at least three non-basic compounds containing nitrogen. They were obtained from the acid insoluble resin from which fats and alkali soluble substances had been removed by treatment with sodium hydroxide. It is, of course, possible that they did not exist as such in the plant but were produced from natural precursors by the alkali treatment. For the time being they are referred to as Compound A ($C_{20}H_{17}O_4N$), Compound B ($C_{20}H_{15}O_4N$), and Compound C ($C_{31}H_{33}O_5N$). The given formulae

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Contribution from the Division of Chemistry, National Research Laboratories, Ottawa, Canada. Issued as N.R.C. No. 1123.

[2] *Chemist.*

are necessarily only tentative and only the first contains methoxyl groups, but the analysis indicates insufficient methoxyl for two such groups. It appears probable that only one methoxyl is present and that an N-methyl group accounts for the uncertain results.

Experimental

The material for the present examination consisted of an entire tree exclusive of the roots. It was obtained through the courtesy of Mr. A. B. Muddiman, Canadian Government Commissioner, Mexico City, to whom sincere thanks are due. The leaves, which weighed 2 kg., were examined separately but the total alkaloid content was very small. The wood and bark were first passed through a rotary plane and the resulting shavings ground in a mill, yielding 20 kg. of material. It was then extracted with methanol in a Soxhlet apparatus.

Chelerythrine

The solvent-free extract was heated with twice its weight of glacial acetic acid until a homogeneous mixture was obtained, diluted with 10 volumes of boiling water and filtered through a layer of charcoal. This procedure was repeated twice. Hydrochloric acid was added to the combined filtrate and the chelerythrine hydrochloride, which separated on cooling, was filtered off. It was recrystallized once from hot very dilute hydrochloric acid, and it then consisted of yellow-brown crystals that weighed 190 gm. (yield, 0.86%). The free base was regenerated from a portion of this by shaking with dilute ammonia in the presence of chloroform. The washed extract was evaporated to a syrup and treated with methanol. The base that separated was recrystallized twice from chloroform–methanol; it then consisted of chelerythrine, melting at 207° C.* Found: C, 69.33; H, 5.70; N, 3.92; OMe, 25.00, 24.83%. Calc. for $C_{21}H_{19}O_5N$: C, 69.04; H, 5.21; N, 3.84; 3 OMe, 25.47%.

The ψ-cyanide of the chelerythrine was prepared by the procedure of Späth and Kuffner (1). After washing with dilute hydrochloric acid it was recrystallized from acetone–methanol. The colourless plates then melted sharply at 261° C., which is the accepted melting point.

Protopine

The filtrate from the crude chelerythrine hydrochloride was evaporated *in vacuo* to remove the water and most of the acetic acid. The residue was extracted with hot water; the extract on cooling deposited a small amount of chelerythrine hydrochloride. The filtrate was basified with ammonia and extracted with chloroform. The residue from the latter was redissolved in hot dilute hydrochloric acid. The cooled solution deposited a little chelerythrine hydrochloride and then on slow evaporation some protopine hydrochloride. The base regenerated from the last and recrystallized melted at 210° C. either alone or in admixture with protopine. The yield was 8 gm.

* *All melting points are corrected.*

Alkaloid P61 and Compound A

The mother liquor from the protopine hydrochloride was again basified with ammonia and extracted with chloroform. The washed extract was evaporated to a small volume and treated with methanol until the incipient turbidity just disappeared on mixing. In the course of several days a small crop of protopine crystals separated. These were filtered off and the solvents boiled out of the filtrate after the addition of dilute hydrochloric acid. There remained a small amount of amorphous halogen-free residue, which was washed with acetone and recrystallized from chloroform–acetone until colourless micaceous plates of Compound A melting at 302° C. were obtained. Found: C, 71.29, 71.19; H, 5.11, 5.26; N, 3.94, 4.02; OMe, 14.35, 14.98, 15.57%. Calc. for $C_{20}H_{17}O_4N$: C, 71.64; H, 5.08; N, 4.18; 2 OMe, 18.50%.

The cooled extract deposited a crop of brilliant yellow needles, which were recrystallized from hot water, in which they are moderately soluble. Alkaloid P61 regenerated from the above hydrochloride was recrystallized from chloroform–methanol; it then melted sharply at 210° C. When admixed with chelerythrine it was completely liquid at 190 to 200° C. Found: C, 69.69, 69.77; H, 5.48, 5.57; N, 3.88, 3.85; OMe, 22.20, 22.20%. Calc. for $C_{21}H_{19}O_5N$: C, 69.04; H, 5.21; N, 3.84; 3 OMe, 25.47%.

Allocryptopine

The filtrate from which the hydrochloride of alkaloid P61 had crystallized was saturated with potassium nitrate. The sparingly soluble nitrates were filtered off, the filtrate was basified with ammonia, and the liberated base extracted with ether. The residue from the washed extract was dissolved in methanol and inoculated with a crystal of allocryptopine. The crystals, which separated immediately, were filtered off and recrystallized once from hot methanol. Allocryptopine as thus obtained melted sharply at 160° C. either alone or in admixture with an authentic specimen. The yield was about 1.0 gm.

Compound B and Compound C

The water insoluble resin from which the alkaloids had been extracted was thoroughly washed, dried, and extracted with ether. The ether extract, as well as the ether insoluble portion, was digested on a steam-bath for 24 hr. with an excess of aqueous sodium hydroxide. The non-saponifiable portion of the ether extract was again extracted with much ether and the filtered and washed solution evaporated. The residue, in contact with methanol–acetone, deposited crusts of brown crystals, which were recrystallized three times from chloroform–acetone. Compound B as thus obtained consisted of orange-brown prisms melting sharply at 191° C. Found: C, 72.05, 72.07; H, 5.05, 4.80; N, 4.26, 4.04%. Calc. for $C_{20}H_{15}O_4N$: C, 72.07; H, 4.51; N, 4.20%.

The unsaponifiable portion of the ether insoluble resin was first washed by decantation and then at the pump. After drying, it was extracted with cold acetone from which some Compound B was obtained. The residue was next extracted with chloroform and the solvent largely distilled from the extract.

Acetone was added until the turbidity just disappeared on mixing. The product that then separated consisted of a mixture of Compound B, together with one much less soluble. The mixture was washed with cold chloroform until the former had largely dissolved. It was then dissolved in a large volume of boiling chloroform and the filtered solution evaporated to a small volume. The crystals that separated were redissolved in hot dioxane and the solution was evaporated somewhat. The cooled solution deposited colourless needles, which were washed with chloroform, acetone, and methanol in the order named. Compound C as thus obtained melted at 332° C., shrinking somewhat at 327° C. Found: C, 74.06, 74.35; H, 6.38, 6.38; N, 2.73, 2.69%. Calc. for $C_{31}H_{33}O_5N$: C, 74.54; H, 6.61; N, 2.80%.

Reference

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THE ALKALOIDS OF *THERMOPSIS RHOMBIFOLIA* (NUTT.) RICHARDS[1]

By Richard H. F. Manske[2] and Léo Marion[2]

Abstract

In addition to 3-methoxy-pyridine, which had previously been isolated from *Thermopsis rhombifolia* (Nutt) Richards, the following known alkaloids have now been obtained—thermopsine, N-methyl-cytisine, and cytisine. Two other alkaloids that are regarded as new have also been obtained. They are now named rhombifoline ($C_{15}H_{20}O_2N_2$) and rhombinine ($C_{16}H_{22}O_2N_2$) and have been characterized as their perchlorates and picrates. The free bases have not been obtained crystalline and the given empirical formulae are regarded as tentative. Incidentally two neutral non-nitrogenous compounds were isolated. They are referred to as Compound A ($C_{19}H_{20}O_{10}$) and Compound B ($C_{22}H_{16}O_6$).

The isolation of 3-methoxy-pyridine from *Thermopsis rhombifolia* (Nutt.) Richards has already been recorded by one of us (1). Since then the greater proportion of the total bases have been obtained crystalline, either as such or in the form of a derivative. The chief alkaloid proved to be N-methyl-cytisine and the next in abundance was thermopsine, an alkaloid first isolated from *T. lanceolata* R. Br. by Orechoff and co-workers (2). A small amount of cytisine was also found, but there was no evidence that *d*-sparteine, anagyrine, or homothermopsine, alkaloids found in *T. lanceolata*, are present in the plant under investigation. No other known alkaloids could be isolated. Two bases, however, were isolated as perchlorates. Neither was obtained in the crystalline condition as free base so that the analytical figures are to be accepted with reserve. They appear to be new and are referred to as rhombifoline and rhombinine. The former is best represented by $C_{15}H_{20}O_2N_2$ and, therefore, does not appear to be identical or even isomeric with any of the 30 or more known papilionaceous alkaloids. Rhombinine is probably $C_{16}H_{22}O_2N_2$ or $C_{16}H_{20}O_2N_2$, neither of which formulae is isomeric with known bases. Both alkaloids are monobasic and yield sparingly soluble, well crystallized picrates.

The acid insoluble portion of the plant was not thoroughly examined but two neutral non-nitrogenous compounds were incidentally isolated. When only a small amount of dilute acid was added to the methanolic extract a thick syrupy aqueous solution was obtained. When the filtered solution was then extracted with ether a sparingly soluble product gradually crystallized from the aqueous phase. This was found to consist of two substances, *A* ($C_{19}H_{20}O_{10}$) and *B* ($C_{22}H_{16}O_6$), which were separable on account of their different solubilities. Substance *A* does not contain methoxyl, but substance *B* contains one such group. No attempt has yet been made to determine the nature of these compounds.

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[2] *Chemist.*

Experimental

The material for the present investigation was collected in the Province of Alberta during and shortly after flowering. No attempt was made to obtain the subterranean shoots and the roots. There was available a total of 200 kg. of dried and ground material. It was extracted with methanol, and the extract was evaporated *in vacuo* and finally freed of the last traces of methanol by passing through it a current of steam. Sufficient water was then added to make a total volume of 100 litres and enough hydrochloric acid to render the solution acid to Congo red. The whole was then heated, cooled, and preserved in a refrigerator for about four weeks. It was then possible to decant the syrupy aqueous solution from the separated resins and filter it through a layer of charcoal with comparative ease.

Substances A and B

The clear aqueous filtrate obtained as described was extracted with several portions of ether. A sparingly soluble product slowly separated from the aqueous phase. After 24 hr. it was filtered off, washed with water, and dried. The mixture was extracted with several portions of hot methanol. This residue was dissolved in hot dioxane, and the filtered solution was diluted with hot water and evaporated somewhat on the steam-bath. On cooling, almost colourless fine needles, melting not quite sharply at 218° C.*, separated. When the substance was dissolved in pyridine and the solution diluted with an equal volume of water, it crystallized in pale yellow stout prisms melting at the same temperature. It is more soluble in a mixture of equal parts of dioxane and water than in either solvent alone. A portion was recrystallized from this mixture and after washing with methanol substance *A* then melted at 218 to 220° C. Found: C, 55.80, 55.75; H, 4.70, 4.87%. Calc. for $C_{19}H_{20}O_{10}$: C, 55.88; H, 4.90%.

The methanolic extract of the above mixture was evaporated to a small volume. The product that separated on cooling was filtered off, washed with a little methanol, and extracted with cold dioxane. The sparingly soluble residue consisted largely of substance *A*. The dioxane extract was diluted with an equal volume of water, whereupon a sparingly soluble substance separated. It was redissolved in warm dioxane and the solution again diluted with water. Substance *B*, which then crystallized, in colourless fine needles melted at 257° C. Found: C, 70.37, 70.30; H, 4.30, 4.22; OMe, 8.06, 7.75%. Calc. for $C_{22}H_{16}O_6$: C, 70.21; H, 4.26; OMe, 8.25%.

Total Alkaloids

The filtered aqueous solution from which compounds *A* and *B* had separated was basified with ammonia and extracted with chloroform until no more extract was obtained. The residue from the chloroform extract consisted of a viscous brown resin. For further purification it was redissolved in dilute hydrochloric acid and the filtered solution exhausted with ether. It was then basified with ammonia and exhausted with chloroform. The residue

* *All melting points are corrected.*

from the chloroform extract then consisted of a pale yellow resin which weighed approximately 500 gm. (0.25%).

Thermopsine

The total crude alkaloid was redissolved in dilute hydrochloric acid, basified once more with ammonia, and then extracted with ether until only traces of alkaloid were obtained. (The aqueous solution was then extracted with chloroform to yield extract *C*.) The residue from the ether extract was dried *in vacuo* and the syrupy residue treated with dry ether and just sufficient methanol to effect solution. When the solution was inoculated with a crystal of thermopsine (which was obtained originally by a repetition of this procedure) immediate crystallization ensued. When it was recrystallized from methanol, thermopsine was obtained in brilliant octahedra melting sharply at 207° C.; $[\alpha]_D^{25} -165.5°$ ($c = 0.996$ in 95% ethanol). Calc. for $C_{15}H_{20}ON_2$: C, 73.77; H, 8.20; N, 11.48%. Found: C, 73.86, 73.85; H, 8.14, 8.32; N, 11.40, 11.56%.

Thermopsine methiodide was prepared according to the procedure of Orechoff *et al.*, who record the melting point as 241 to 242° C. It melted at 250° C. (243° C. uncorr.) with effervescence. The above properties are those of thermopsine but the picrate is stated to melt at 208 to 209° C. The picrate was prepared in methanol and recrystallized from the same solvent. It melted sharply at 262° C. In spite of this single discrepancy the base is regarded as identical with thermopsine. The total yield of thermopsine was 95 gm. (0.048%).

It was from the filtrate from the thermopsine that the isolation of 3-methoxypyridine was effected.

N-methyl-cytisine

The chloroform extract *C* (*vide supra*) was completely freed of solvent and moisture by heating *in vacuo*. It was finally distilled *in vacuo* (0.1 mm.) and two main fractions were collected (up to 210° C. and 210° to 250° C.). The temperatures are those of the surrounding oil-bath.

The lower boiling fraction was treated with 25% of its volume of acetone and inoculated with a crystal obtained in a preliminary experiment. The fine colourless needles that separated immediately were filtered off, washed first with acetone–ether, and then with ether. The base then melted at 135 to 136° C. A portion was recrystallized from acetone–ether. N-methylcytisine as thus obtained consisted of brilliant colourless prisms melting sharply at 138° C. Calc. for $C_{12}H_{16}ON_2$: C, 70.59; H, 7.84; N, 13.41%. Found: C, 70.84, 70.44; H, 7.73, 7.79; N, 13.38, 13.37%. For further authentication a specimen of N-methyl-cytisine was prepared from cytisine (from *Cytisus laburnum* L.) by the method of Partheil (3). The base was distilled *in vacuo* and recrystallized from acetone–ether. It melted sharply at 138° C. either alone or in admixture with the base from *T. rhombifolia*. The perchlorate, which is sparingly soluble even in hot methanol, was obtained in brilliant colourless prisms melting not quite sharply at 282° C. The total

yield was 214 gm. (0.107%). N-methyl-cytisine picrate was prepared and recrystallized from a mixture of acetone and methanol from which it separated in pale yellow flat needles, melting at 193° C.

Cytisine

The fraction boiling at 210° to 250° C. deposited some thermopsine, which was filtered after the addition of a little acetone. The filtrate then deposited another base, which was filtered off and washed with acetone–ether and then with ether. When this was recrystallized from acetone, cytisine was obtained in brilliant pearly plates melting sharply at 156° C. A specimen of cytisine was prepared from *Cytisus laburnum* L. It was first crystallized from chloroform–ether, then distilled *in vacuo*, and recrystallized from acetone. It also melted at 156° C. and a mixture of the base from the two sources melted at the same temperature. Calc. for $C_{11}H_{14}ON_2$: N, 14.75%. Found: N, 14.37, 14.34%. The perchlorate is sparingly soluble in methanol and melts at 300° C. The total yield of cytisine was 18 gm. (0.009%). The picrate of cytisine, after recrystallization from boiling water from which it separated as yellow flat rhombs, melted at 302° C.

Rhombifoline

The filtrate from which the thermopsine had crystallized was distilled *in vacuo*. At first the 3-methoxy-pyridine was obtained and then an intermediate fraction was collected. This was treated with perchloric acid in methanol. The perchlorate thus obtained was a mixture. It was first extracted with hot methanol, then recrystallized from hot water, and finally recrystallized from a large volume of boiling methanol. The perchlorate of rhombifoline thus obtained consisted of brilliant stout prisms melting sharply at 242° C. Found: C, 50.00, 50.19; H, 5.86, 5.97; N, 7.73, 7.91, 7.69%. Calc. for $C_{15}H_{20}O_2N_2.HClO_4$: C, 49.94; H, 5.83; N, 7.77%.

The free base was regenerated from a portion of the perchlorate. It could not be obtained crystalline. The picrate is virtually insoluble in cold methanol. It was recrystallized from the boiling solvent; it then melted at 207° C. Found: C, 51.60, 51.76; H, 4.99, 4.88; N, 14.36, 14.21%. Calc. for $C_{21}H_{23}O_9N_5$: C, 51.53; H, 4.70; N, 14.32%. The total yield of perchlorate was 44 gm. (0.022%). The methanolic mother liquor from the above perchlorates yielded some thermopsine. The perchlorate of the latter is very soluble in most solvents, including acetone, and could in fact not be obtained in a crystalline condition, even when prepared from the pure base.

Rhombinine

The highest boiling fraction from the thermopsine filtrate yielded some crystalline thermopsine and an acetone filtrate on appropriate treatment. This was combined with the higher boiling fraction from the chloroform extract (*C*), from which some thermopsine was also recovered. The mixture was then treated with perchloric acid in acetone. The virtually insoluble perchlorate was washed with acetone and recrystallized several times from a large volume of hot methanol. The colourless needles of rhombinine per-

chlorate thus obtained melted fairly sharply at 313° C. when placed in the bath at 305° C. Found: C, 52.10, 51.95; H, 5.87, 6.02; N, 7.57, 7.51%. Calc. for $C_{16}H_{22}O_2N_2.HClO_4$: C, 51.27; H, 6.14; N, 7.47%.

The free base could not be obtained crystalline. The picrate was recrystallized from methanol–acetone, and it consisted of pale yellow prisms virtually insoluble even in hot methanol. It melted sharply at 253° C. Found: C, 53.02, 53.09; H, 4.87, 4.88; N, 14.33, 14.50%. Calc. for $C_{22}H_{25}O_9N_5$: C, 52.48; H, 4.97; N, 13.92%. The total yield of rhombinine was 8 gm. (0.004%).

During the process of examining the various fractions there was accumulated a considerable amount of impure perchlorates and mother liquors. These were worked up systematically as such or combined and reworked until the yields given were obtained. Finally there was left a residue of about 6 gm. of mixed perchlorates and some 30 gm. of not fully purified bases. In all cases the essential constituents of these mixtures were known to be alkaloids already described. For example a mixture of cytisine and N-methyl-cytisine can be separated only with difficulty but nevertheless fairly completely. If the mixture is dissolved in a little acetone, cooled, and inoculated with cytisine only this base crystallized at once. If the filtrate is then concentrated and treated with dry ether the N-methyl-cytisine can be largely separated. Either base can then be purified by recrystallization and the combined mother liquors can be reworked in the same way, particularly if the bases are first redistilled. An exhaustive examination of the various fractions failed to disclose the presence of other bases in significant amounts. There was a very small fraction of a higher boiling base that could not be obtained crystalline either as such or as a derivative.

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